Florence-Utah Symposium corner: from genetics toepigenetics of male infertility

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EDITORIAL

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The etiopathogenesis of testicular failure remains unknown in about half of the clinical cases and it is referred to as 'idiopathic infertility'. Since the number of genes involved in human spermatogenesis is possibly over thousands and only a small fraction has been identified and screened for mutations in infertile men (Nuti & Krausz, 2008), it is likely that a substantial proportion of 'idiopathic' testicular failure is of genetic origin.

Several new strategies are now available for searching spermatogenesis candidate genes based both on animal models and on human studies, and a great selection was showcased at the 3rd Florence-Utah Symposium (14–16 September, 2007). M.A. Handel presented data generated in the context of the ReproGenomics Program which deals with genome-wide mutagenesis in the mouse followed by phenotype-driven identification of mutations that cause infertility (http://reproductivegenomics.jax.org). Proteomics and transcriptomics approaches also open new horizons although their diffusion is limited by the complexity and the high cost of such analyses (Platts *et al.*, 2007; Aitken & Baker, 2008; Oliva *et al.*, 2008). In recent years, case/control association studies dealing with polymorphisms have become more and more popular among human geneticists aiming to identify genetic 'risk factors'. However, despite the general enthusiasm towards genetic variants, many in the field are often facing frustrating situations in which initial promising data are not confirmed in later studies (Krausz & Giachini, 2007). A rather unique example of successful replication of a previously reported genetic risk factor for impaired spermatogenesis, on a substantially larger study population than the original one, concerns the gr/gr deletion of the AZFc region (Repping *et al.*, 2003; Giachini *et al.*, 2005, 2008).

The most frequently encountered biases responsible for discrepancies between association studies are inadequate sample size and inappropriate selection of patients and controls. If the purpose of a case–control association study is to detect the effect of a genetic variant on spermatogenesis, the appropriate control ('disease-free') group should be represented by normozoospermic men rather than men from the general population (7% are infertile) or fertile men with unknown sperm count (10% of 'fertile men' are severely oligozoospermic). Patients also should be properly selected because the association between a genetic risk factor and spermatogenic failure may be weakened or lost by the inclusion of patients with known causes of spermatogenic failure. Both the Repping *et al.* (2003) and the Giachini *et al.* (2008) studies provided strong support to the importance of ethnic and geographic matching of patients and controls (especially relevant for Y chromosome variants).

The best approach to minimize the effect of confounding factors would be the use of high-throughput genotyping (a rapid analysis of hundreds of thousands of SNPs) in large study populations. Unfortunately, genome-wide association studies are still lacking in the field of andrology. There is an urgent need for this type of studies not only to improve our knowledge of genetic risk factors but also to provide a strategy that would accelerate the identification of spermatogenesis candidate genes. The review article by Rodriguez-Murillo and Greenberg (2008) focuses on the strengths and weaknesses of association studies and provides general indications/knowledge for this type of genetic analysis.

The second review article by Carrell *et al.* (2008) deals with male infertility related to sperm protamine abnormalities. Abnormal protamine replacement may lead to generally diminished sperm quality, impaired fertilization ability and increased DNA damage (Aoki *et al.*, 2006). The aetiology of sperm protamine abnormalities (expressed as abnormal protamine 1 : protamine 2 ratio) is largely unknown although some recent data appear to provide previously unappreciated clues. The sequencing of genes relevant to protamine expression in different laboratories did not identify clear cut cause–effect mutations so far. On the other hand the analysis of polymorphic sites in the Contrin (YBX2) gene (a transcription factor and translation repressor) revealed the presence of a few SNPs possibly involved in abnormal protamine expression (Hammoud *et al.*, 2008). More studies are needed in this specific area of research, while it would be especially useful to combine analysis of multiple polymorphisms with environmental factors. Besides mutational screening, the authors discuss preliminary data generated by sperm mRNA profiling that show consistent variation of the sperm transcriptome between men with specific protamine defects and controls. Finally, they focus on the link between epigenome (histone retention and DNA methylation) and chromatin compaction defects. Especially important is the hypothesis related to the role of 'programmed' histone retention in sperm chromatin which should ensure specific marking of genes to be

preferentially activated in early embryo development. Abnormal protamine replacement could therefore affect not only gene expression in mature sperm but also in early embryo and ultimately affect embryo implantation capacity.

These studies are in keeping with the notion that the transcriptional machinery who governs the differentiation programme of male germ cells is highly specialized and intimately connected to unique epigenetic control (Kimmins & Sassone-Corsi, 2005). The epigenetic programme of male germ cells involves the generation of highly specific histone variants, the histone-to-protamine replacement process and the activation of distinct histone modifications that are uncommon in somatic cells. In addition, recent evidence indicates that the microRNA pathway plays a critical role in the timing of post-meiotic RNA translation. The chromatoid body of spermatids, a unique organelle with remarkable structural and functional features, seems to operate as a 'nerve-centre' for the RNA metabolic pathway, in a fashion that is reminiscent of the P-bodies of somatic cells (Kotaja & Sassone-Corsi, 2007).

The multiple fascinating facets of germ cell development make it unique for biologists and clinicians. The challenge of future studies lies on linking the interplay of all molecular mechanisms to the various pathologies, specifically in the understanding of how genetic regulation and epigenetic control operate in concert to insure the fidelity and efficacy of the differentiation programme.

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